1964

Studies on the Induction of Swarming in Tylenchorhynchus Martini, Fielding, 1956 (Nematoda, Tylenchida).

Jere Mac Mcbride
Louisiana State University and Agricultural & Mechanical College

STUDIES ON THE INDUCTION OF SWARMING IN *TYLENCHORHYNCHUS MARTINI* FIELDING, 1956 (NEMATODA, TYLENCHIDA)

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Botany and Plant Pathology

by
Jere M. McBride
B.S., Louisiana Polytechnic Institute, 1959
M.S., Louisiana State University, 1961
January, 1964

ACKNOWLEDGMENTS

The writer extends grateful acknowledgments for the helpful guidance and direction of the research work and for generous assistance in the preparation of this manuscript to Dr. John P. Hollis; to Dr. Wray Birchfield for suggestions and encouragement during the course of this work; to Dr. F. Ben Struble of Oklahoma State University for cultures of Tylenchorhynchus martini and T. brevidens; to Dr. R. A. Chapman of the University of Kentucky for cultures of T. claytoni and T. maximus; and to Dr. S. J. P. Chilton for providing facilities for this work.

TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

Swarming of nematodes in water occurs on contact between individuals possessing a sticky condition of the cuticle. Nematodes in this condition adhere to one another, forming aggregations of specimens in a state of exaggerated activity, characterized by jerky movements which result from restrictions imposed on the movement of individual nematodes.

This work is an extension of preliminary investigations which indicated that the swarming condition in Tylenchorhynchus martini Fielding, 1956 is the result of an altered physiological state induced under conditions of rapid and abundant host-plant growth in greenhouse pots. Host nutrition tests indicated that complete nutrition of the host with all essential nutrient elements was necessary to the induction of swarming. Nutrient supplements of acid casein hydrolysate, asparagine, and glutamine had no effect on induction of swarming in cultures provided with abundant and essential mineral nutrients.

Additional evidence on specificity of the swarming reaction was obtained from the demonstration that T. martini and T. claytoni Steiner, 1937 swarmed together. Combined with the fact that T. martini and an unidentified species of Tylenchorhynchus swarmed separately in mixtures, these results indicate that both species and group specificity of the swarming reaction may be common in the genus

Tylenchorhynchus. It is of interest that morphologically T. martini and T. claytoni are close relatives, whereas the Grand Isle Tylenchorhynchus is morphologically distinct from these species.

Additional evidence was compiled on the nature of the swarming reaction. Tests confirmed previous results that all stages of nematode development were involved in swarms of T. claytoni, and that reactions between the different sexes in this species and of individuals of both sexes with females of T. martini were not conditioned by the sex of the individuals.

Field samplings have shown that swarming populations of some species of nematodes may occur commonly; however occurrence of the phenomenon is very rare in populations of T. martini from fields of Louisiana rice and sugarcane, and is of short duration whenever encountered.

The swarming reaction has been reported in populations of 13 species of plant-parasitic and free-living nematodes, embracing all Subclasses of the Class Nematoda.

INTRODUCTION

The phenomenon of swarming was first reported in the nematode Tylenchorhynchus martini Fielding, 1956, in 1958 (9), and several short reports were published subsequently on its mechanism, induction, and nature (10, 11, 14).

Swarming of nematodes in aqueous suspensions occurs on contact between individuals possessing a sticky condition of the cuticle. In contrast to normal, nonswarming nematodes, populations of nematodes in this physiological condition adhere to one another, forming aggregations in a state of exaggerated mobility. The rapid, jerky motions are the result of restrictions on the movements of individuals by other individuals in the swarm.

Although swarms have been observed in 13 species of nematodes, they occur sporadically, and in the most favorable subject for study (T. martini) they appear to result from, or to be induced by, unknown nutritional and genetic factors. The object of this investigation was twofold: (a) to determine whether complete nutrition of the host by all essential mineral elements influenced the induction or onset of swarming in T. martini, and (b) to obtain additional evidence on the specificity of the swarming reaction in the genus Tylenchorhynchus. These problems are of primary importance because they block progress toward an understanding of the nature and role of the phenomenon of

swarming. Associated factors such as the relation of swarming to other manifestations of altered physiological state in nematodes and, in particular, to pathogenicity of plant-parasitic nematodes are perhaps of equal importance; but it was evident at the outset that their eventual solution would be dependent upon the ability of future investigators to reproduce and manipulate the swarming condition. It is for this reason that the results reported in this thesis deal exclusively with the induction and specificity of the swarming reaction in one species of plant-parasitic nematode.

REVIEW OF LITERATURE

The first description of swarming in nematodes was by Hollis (9) in 1958, when the phenomenon was observed in an aqueous suspension of greenhouse-bred Tylenchorhynchus martini Fielding, 1956. With the exception of 3 reports (18, 26, 29), all accounts of swarming have emanated from the Plant Pathology Laboratory of Louisiana State University (9, 10, 11, 12, 14). These later reports pertained to the mechanism, induction, and nature of the swarming reaction.

Results of preliminary investigations revealed swarming to be dependent upon a sticky condition of the nematode cuticle (10, 11). Active masses of nematodes, exhibiting rapid, jerky movements, are formed in aqueous suspensions as a result of cuticular stickiness.

Nematodes possessing the sticky cuticle apparently attain such a condition by virtue of an innate change in the structure of the exocuticle. Swarming was inhibited in nematodes incubated in solutions of trypsin or other enzymes containing endopeptidases, and this inhibition was removed when the nematodes were washed free of the enzymes in distilled water (11, 13). It is still not clear whether this inhibition is enzymatic, or if it is due to a masking of the cuticle by the enzyme due to electrostatic attraction (13).

Swarming was found dependent upon nematode movement, population density and time. Swarms resulted to an appreciable

extent only when sufficient density and movement occurred. In a limited series of tests, swarms were formed as the density of nematodes increased from 0.2 nematode per square mm (13). At less than 0.1 nematode per square mm, swarming was partial and incomplete, and did not progress to large swarms even with time. Swarming in aqueous suspensions was found to be independent of pH; thus at pH levels from 3 to 11 neither swarming nor the viability of nematodes were affected (9, 10, 13). Swarming in water was inhibited at pH 2.25, presumably because the nematodes were rendered quiescent in a short time, although they were not killed in less than 24 hours.

Induction of swarming in greenhouse-bred populations required 6 to 10 weeks under the favorable conditions of rapid and abundant host plant growth (14).

Extensive tests conducted on swarming revealed the reaction to be independent of a large number of chemical treatments, including alkalis and acids in water solutions (10, 13). Swarming was affected by some of the treatments, but only as a consequence of toxicity.

The phenomenon occurred independently of light and of all temperatures under which swarming nematodes exhibited mobility. Temperature became a factor only when the movement or viability of the nematodes was affected. Incipient swarms were formed at 5°C, provided the population density of nematodes was sufficient, and a few minutes elapsed before temperature equilibrium was established (13).

All stages of nematode development apparently participate in swarming. Swarms of Rotylenchulus sp. were found to consist of pre-adults, whereas swarms of all other nematodes were observed to consist of all stages, including both sexes (13). The swarming reaction of an undescribed species of Tylenchorhynchus from Grand Isle, Louisiana was nonsexual because both males and females swarmed together. In nematode species where only females occur, such as T. martini, swarms were composed of the one sex.

Observations of swarming were reported for both plant-parasitic and free-living nematodes (10, 13, 18, 29). Among the diverse genera observed swarming were Rotylenchulus, Mononchus, Hemicycliophora, and Dorylaimus.

Nematodes in the swarming state were not dependent upon a nutrient supply to maintain their swarming ability (13). Swarming persisted in nematodes for long periods in the absence of food with populations apparently reverting to the nonswarming conditions only when food again became available. As the ancestral population declined it was replaced by a population of nonswarmers at lower levels of nutrition.

Swarming has been found independent of all common soil environmental factors including crop and soil type. Purity of the soil population of the swarmers, the percentage of swarmers in the total

population of nematodes extracted from the soil, and adverse conditions in the soil, such as drying, had no effect on the phenomenon,

Swarming bears a superficial resemblance to a phenomenon known as anabiosis. Anabiosis, meaning reanimation after apparent death, refers to a dormant condition which many nematode species are capable of entering. Aggregations formed by nematodes in the dormant state are referred to as "wool," "eelworm wool," or "curd (24)." Anabiosis and swarming differ fundamentally in their modes of induction and nature (11). The anabiotic condition is induced by adverse environmental conditions, and consists of aggregations of preadult nematodes in a dormant state. In contrast to this, swarming is induced by rapid and abundant host-plant growth, and is characterized by masses of nematodes in a state of activity and all stages of development.

Swarming was found to be independent of variations in all common stimuli such as light, temperature and pH, in contrast to a number of similar phenomena (28) and the "swarming" of a Rhabditis sp. reported by Staniland (22) that was induced by light. This occurred without evidence of a cuticular stickiness, and was controlled completely by altering light intensity.

Specificity of the swarming reaction suggests that the ability of a nematode to respond to factors inducing swarming is under genetic control. We have been led to suspect genetic differences in populations of T. martini from time to time by the variable results obtained

in greenhouse swarming induction tests. Under the most favorable conditions for the induction of swarming, certain populations of T. martini have swarmed, and others have not swarmed.

Swarming has been observed in a limited number of nematode genera and species under field and greenhouse conditions (Table 1). Included in the list of swarmers are Tylenchorhynchus martini Fielding, 1956; Tylenchorhynchus claytoni Steiner, 1937; Tylenchorhynchus sp. from Grand Isle, Louisiana; Dorylaimus pusillus Cobb, 1893; larvae of Rotylenchulus reniformis Linford and Oliveira, 1940; larvae of Rotylenchulus sp.; Hemicycliophora typica deMan, 1921; Hemicycliophora spp., Scutellonema sp., Mononchus sp., and Helicotylenchus nannus Steiner, 1945.

Swarming of plant-parasitic nematodes was noted by Whitehead (29) in Kenya. Although the species were not mentioned, it was learned that the swarmers were species of Hemicycliophora, Rotylenchulus, and Scutellonema (13). Meyl observed swarming--the clumping of both sexes of H. typica in brackish, German soils and referred to the phenomenon as "Nesterbildung" (18). Steiner (26) reported swarming in an Oncholaimus species in Puerto Rico. Greenhouse populations of swarming T. claytoni were observed by Chapman (3) in Kentucky. A Mononchus sp. and a Hemicycliophora species similar to H. typica, taken from several different grasses in Kenya, were observed in a swarming condition (12). In a letter, Lees (17) reported that a

phenomenon resembling swarming was observed in vinegar suspensions of Panagrellus silusiae (deMan, 1913) Goodey, 1945, and that it was induced by adding to the suspensions of nematodes, fibers of filter paper, which acted as centers around which the swarms occurred. Most reports of swarming have emanated from the Plant Pathology Laboratory at Louisiana State University (Table 1).

Swarming, in general, was once thought to occur only in greenhouse-bred populations of T. martini (11), but observations over a period of years and other reports (Table 1) now indicate that it also occurs rarely under field conditions.

Swarms of nematodes undoubtedly occur in the soil about the roots of plants, but such swarms, excepting those seen by Meyl (18), have not been detected in isolations from soil and have not been demonstrated to actually occur outside of water suspensions in the laboratory.

Table 1. Reported observations to the end of 1963 of the phenomenon of swarming in nematodes.

Nematode	Location	Inducing Site	Reference	Observer
Tylenchorhynchus				
martini	Louisiana	Greenhouse	9	L. S. U.
martini in mixed population with T. ewingi	Louisiana	Field	13	L. S. U.
martini	Louisiana	Flooded rice field	13	L. S. U.
Tylenchorhynchus				
claytoni	Kentucky	Greenhouse	3	Chapman
Tylenchorhynchus sp. from Grand Isle, La.	Louisiana	Greenhouse	11	L. S. U.
Hemicycliophora				
typica	Germany	Field	18	Meyl
Hemicycliophora sp.	Kenya	Field	12,29	Whitehead & Hollis
Hemicycliophora sp.	Louisiana	Greenhouse	11	L. S. U.
Scutellonema sp.	Kenya	Field	13,29	Whitehead & Hollis
Rotylenchulus sp.	Kenya	Field	13,29	Whitehead & Hollis
Rotylenchulus reniformis (larvae)	Louisiana	Field	11	L. S. U.
Monochus sp.	Kenya	Field	13	L. S. U.
Oncholaimus sp.	Puerto Rico	Field	26	Steiner
Dorylaimus pusillus	Louisiana	Greenhouse	11	L. S. U.

MATERIALS AND METHODS

Greenhouse Cultures

Nematode populations were established in the greenhouse for the purpose of inducing swarming in the populations by allowing them to feed on favorable hosts grown in clay pots of sterilized and non-sterilized field soils.

Sterilization of field soils used in culture work was accomplished by steam treatment under pressure, or by fumigation with methyl bromide (CH_3Br). Steam-sterilized soils were autoclaved at 15 pounds pressure for approximately 5 hours. Fumigation of soils was accomplished by placing 400-500 pounds of field soil in a tank. The tank was covered with a plastic sheet, and the soil was treated with two pounds of methyl bromide for 48 hours. Clay pots were cleaned and steam treated under pressure before being used in culture work.

Inoculations were made after plant emergence or at the time of the planting of the seed. Small vials filled with water contained the nematodes, and this inoculum was introduced into the pots simply by breaking the soil surface around the plant roots and pouring the contents of the vials into the pots.

Procedures for Field Sampling

Random soil samples were taken in many parishes of the State

of Louisiana in an effort to collect different species of the genus Tylenchorhynchus in a swarming or nonswarming condition. Samples were obtained from field crops, garden crops, pastures, woodlands, and field of indigenous weeds.

Sampling supplies consisted of pint plastic bags and a sampling tube with a 3/4" internal diameter. Six to 8 soil borings, 6-8 inches in length, were placed into each labeled plastic bag, and these were then taken to the laboratory and processed according to a method described by Seinhorst (20),

Tests for the Specificity of the Swarming Reaction

Swarming populations of T. martini were mixed with swarming populations of T. claytoni to determine if swarming in the 2 species was specific or nonspecific. Greenhouse-bred populations of nematodes were used in each case. The numbers used in the reaction tests varied from 1-5 individuals of each species.

Design of Swarming Induction Tests

Five nutrition tests were designed to evaluate the influence of major and minor nutrient elements in induction of swarming in T. martini. Basic preparations for each experiment, I through V, were essentially the same except for variation in the type and number of treatments, preparation of the various nutrient solutions, and fertilization rates.

One-gallon glazed crocks, with drain holes flush with the bottom, were used in all 5 tests. Fine filter media sand was used as the culture medium throughout the tests. Glass wool was placed over the drain holes to keep the sand from washing out and to facilitate drainage. All crocks were filled with sand to a level of approximately 1/2-inch from the top, flooded with distilled water several times, and planted with rice variety "Bluebonnet 50." The sand and crocks used in Test IV were cleaned with a weak, aqueous solution of sulfuric acid. Nonswarming T. martini populations from field soil were used in all tests, and were added to each crock after germination and emergence of the rice.

The composition of the nutrient solutions employed in Tests I, IV, and V were originally described by Johnson, et al. (16). Chemicals used in the preparation of the solutions were potassium nitrate, calcium nitrate, ammonium phosphate, magnesium sulfate, potassium chloride, boric acid, manganous sulfate, zinc sulfate, cupric sulfate, and ammonium molybdate. Solutions of the 4 salts included in the complete nutrient solution were potassium nitrate, calcium nitrate, ammonium phosphate, and magnesium sulfate, and these were prepared individually at molar concentrations. Other chemicals supplying major elements for incomplete solutions were calcium phosphate (0.01M), calcium sulfate (0.01M), and potassium sulfate (0.5M). The micronutrient solution used in all tests was prepared separately and 1 ml of this solution was added to each liter of the final nutrient

solution. Iron was added separately as ferrous sulfate to the final nutrient solution at the rate of 2 ml per liter. Stock solutions prepared for use in Test IV were purified by methods described by Steinberg (23) and Stout and Arnon (27).

Preparation of the macronutrient solutions for Tests II and III followed the procedures described by Gallegly and Walker (7). Compounds used in the preparation of the complete nutrient solution were calcium nitrate, potassium nitrate, potassium phosphate, magnesium sulfate, calcium chloride, sodium nitrate, potassium chloride, sodium phosphate, and sodium chloride. Salts were made up individually in molar concentrations.

Nutrient-supplement solutions acid casein hydrolysate, asparagine, and glutamine were prepared separately and added to the respective nutrient solutions in Tests II, III, IV, and V.

All experiments were sampled for nematodes at pre-determined intervals by removing approximately 1/2 or 1 pint (473 ml) of the sand medium to a plastic bag. Each sample was processed according to the method previously mentioned.

A soil mixture was used in 2 tests involving nitrogen fertility of rice and sugarcane. This mixture included Sharkey Clay, Olivier Sandy Loam, Grand Isle Sand, and the fine filter media sand used in previous tests. This mixture was steam-treated before use.

An 80 or 90-pound portion of the air-dried, screened soil

mixture was weighed, and then placed in a Number 2 wash tub so that the proper amount of fertilizer could be mixed with it. The fertilizer added depended upon the treatment, and its quanity was based on the 2 million pounds of soil calculated as the weight per acre in a 6-inch plow furrow slice.

Commercial fertilizers were used as the source of mineral nutrition for the plants in these tests. The commercial nitrogen fertilizer contained 16 per cent nitrogen, and a 0-20-20 mixture of phosphorus and potassium was used to supply these elements as required. When phosphorus or potassium was omitted, a 60 per cent fertilizer containing only one of these elements was used. Rice seed was germinated in Petri dishes on moistened filter paper and then transplanted to greenhouse pots. The seedlings were watered for a short time after planting by spraying a fine mist of water over the plants and soil with a Hudson sprayer. This watering method was continued until the plants were anchored well enough to allow normal watering by a hose.

Sugarcane seedpieces of variety 44-101 were germinated in greenhouse flats containing a mixture of soil, sand, and peat moss in the proportions of 3-2-1. After germination, the plants were selected and distributed across the several replicates according to size and transplanted to the greenhouse pots.

After transplanting of the rice and sugarcane, known numbers of nonswarming T. martini were added to each pot. Sampling of the

experiments were commenced 60 days after the nematodes were added and then was continued at weekly intervals for a period of 20 weeks.

Design of Tests of Duration of the Swarming Condition

A test was set up to study the duration of the swarming condition in T. martini in the absence of host plants, and under the influence of minimum growth of host plants.

Host plants in 6 pots containing T. martini swarmers were killed with varsol. Three cultures were kept moist during the experiment by frequent watering, and 3 cultures were permitted to dry. Samplings were made at intervals of 2 months for a period of 8 months and then at monthly intervals for 4 months. Samples were taken by stirring the soil thoroughly and then removing approximately 1 pint of the soil from each pot. At the end of 12 months, the remaining soil was re-potted in 10-inch pots. One rice seedling was transplanted to each pot and sampled at monthly intervals for a period of 4 months.

RESULTS

Greenhouse Cultures

Preliminary work involved attempts to establish and maintain greenhouse cultures of several species of Tylenchorhynchus and certain other genera of nematodes with the ultimate intention of conducting tests on inductions of swarming. The techniques involved in establishing cultures were aimed at obtaining pure populations, or increasing a desired species in mixed populations. Approximately 200 attempts were made to establish and maintain cultures by the 3 methods listed below:

1. Fifty to 100 specimens of the same species were hand-picked and added to sterilized soil planted with a favorable host.
2. Nematodes of mixed populations were extracted from field soil and added to pots of sterilized soil planted with a favorable host.
3. Field soil containing mixed populations were potted and planted with a favorable host.

Nematodes used in attempts to establish greenhouse cultures included Tylenchorhynchus martini Fielding, 1956; T. claytoni Steiner, 1937; T. ewingi Hopper, 1958; T. acutus Allen, 1955; T. maximus Allen, 1955; T. brevidens Allen, 1955; two undescribed

species of Tylenchorhynchus, one each from Grand Isle and Calhoun, Louisiana, respectively; Hemicycliophora sp.; Helicotylenchus nannus Steiner, 1945 and Rotylenchulus reniformis Linford and Oliveria, 1940.

Hand picking a population of 50 to 100 nematodes was the most difficult method of establishing cultures of nematodes in the greenhouse. A few attempts were successful in increasing populations of nematodes to moderate numbers, but none of these were transformed to the swarming condition (Table 1).

Specimens of T. claytoni added to pots of sugarcane increased in number, but did not swarm. A Tylenchorhynchus species from the root zone of peaches at the North Louisiana Agricultural Experiment Station, Calhoun, Louisiana also increased to moderate numbers after hand-picked specimens were added to pots of sterilized soil planted with corn. Hemicycliophora sp. from Kenya also increased after a small number of swarming individuals were added to a pot planted with Rhodesgrass (Chloris gayana Knuth). The nematodes were found swarming a few months after inoculation, but eventually reverted to a nonswarming condition, or the swarming condition was lost, although the population was maintained at a relatively high level. An undescribed species of Tylenchorhynchus commonly found at Grand Isle, Louisiana increased and was maintained in low numbers on sugarcane and corn when hand-picked populations were added to pots of sterilized soil.

Extracting nematodes from field soil and adding the mixed populations as mass inocula to steam or methyl bromide-treated soil was a successful method of increasing populations, but not in inducing the swarming of nematodes. Nematode populations that increased to high numbers but never swarmed included such species as T. martini, Tylenchorhynchus sp., and Hemicycliophora sp. (Table 1).

Populations of T. claytoni, T. sp., R. reniformis, and H. nannus in field soil increased and were maintained in high numbers when field soils containing the nematodes were potted and planted with a favorable host (Table 1). On some occasions, free-living and other plant-parasitic species increased greatly and tended to crowd out the desired species. Field soils often contained diverse genera and the ability of the genera to survive under greenhouse conditions varied.

One swarming population of nematodes was obtained when field soil was potted and planted with a suitable host. Soil from a strawberry field near Hammond, Louisiana was placed in 10-inch pots and planted with 2 strawberry plants taken from the same field. Swarming of T. claytoni occurred 6 months later, but the condition persisted only for a short time, after which Meloidogyne sp. increased and T. claytoni numbers decreased. Attempts to maintain a culture of swarming T. claytoni therefore were not successful.

The nematode genera and species listed in Table 2 represent successful attempts to increase populations. Many additional attempts were unsuccessful, as were attempts to increase population

Table 2. Nematodes which increased in populations on host plants in greenhouse cultures.

Nematode species	Geographical origin	Original host	Host on which tested
Tylenchorhynchus martini	Oklahoma	Grass	Common Bermuda
			Oats
			Sugarcane
Tylenchorhynchus claytoni	Hammond, La.	Strawberry	Strawberry[a]
Tylenchorhynchus sp.	Calhoun, La.	Peach	Corn
Tylenchorhynchus sp.	Grand Isle, La.	Sugarcane	Sugarcane
		Native grasses	Corn
			Corn
			Sugarcane
Tylenchorhynchus sp.	Bastrop, La.	Cotton	Beans
			Cotton
Hemicycliophora sp.	Kenya	Rhodesgrass	Sugarcane
			Rhodesgrass
Helicotylenchus nannus	Hammond, La.	Soybean	Soybean
			Strawberry
Rotylenchulus reniformis	Bunkie, La.	Cotton	Cotton

[a]Swarms found

levels of other species of Tylenchorhynchus. Tylenchorhynchus species which failed to survive and to increase in populations under greenhouse conditions were T. acutus, T. brevidens, T. ewingi, and T. maximus.

Field Sampling

Three populations of swarming T. martini were found under field conditions by the writer during the course of this work. In July, 1962, soil samples were taken from the rice area near Kinder, Louisiana. One of these samples yielded a large swarming population of T. martini. The sample was taken from under common bermudagrass (Cynodon dactylon (L.) Pers.) growing near the edge of a flooded rice field. One month later, additional samples were taken from this same area, but no swarmers were found.

Swarming T. martini were found in 2 soil samples taken during a nematode survey of farms near Hammond, Louisiana in October, 1962. One population came from a field covered with weeds and grasses. It was of interest that this field had previously been planted with strawberries, but later abandoned because of several crop failures. Nematodes taken from this area did not form large swarms until they were concentrated into a small volume. In the counting dish, small swarms of 10-12 nematodes were observed. Other genera including Helicotylenchus, were present, but gave no indication of swarming. Another population of swarming T. martini was taken from a pasture site 3 miles east of Hammond, Louisiana. Swarms

from this population were weaker than those taken from the field previously mentioned. Small swarms of 3 and 4 nematodes were observed. Through manipulations with a needle, it was definitely established that these nematodes possessed sticky cuticles and were swarming. After these nematodes were allowed to feed on sugarcane for several weeks in greenhouse pots, they returned to the nonswarming condition.

Swarming T. martini from 2 different fields and from a greenhouse population were placed together with positive results. There was swarming between individuals from the 2 fields and from the greenhouse cultures. This constitutes evidence that swarming occurs between populations of the same species from different areas.

Soil samples taken from the Carl Drude farm near Hammond, Louisiana contained high numbers of H. nannus. Many of these nematodes were aggregated in small swarms of 10-15 specimens. Approximately 50-60 of these swarms were hand-picked and mixed in a small dish to form larger swarms. Additional soil samples were taken from the same field 30 days later, and again small swarms were found.

Soil from the Carl Drude farm was placed in greenhouse pots and planted with strawberry and soybean. Populations of H. nannus were hand-picked also and added to sterilized soil, and this soil was planted with the same two crops. The nematode populations were maintained in field soil, but their ability to swarm was lost. Hand-picked individuals in the sterilized soil treatments did not survive.

This is, to the writer's knowledge, the first report of swarming in the genus Helicotylenchus.

Swarming Reaction Tests

Swarming individuals of T. martini and T. claytoni were mixed to determine whether they would swarm together or separately. A characteristic utilized to evaluate the possible influence of sex on swarming was the abundance of males present in T. claytoni and their absence in T. martini. The combinations of nematodes were as follows:

T. claytoni ♀	T. martini ♀
T. claytoni ♂	T. martini ♀
T. claytoni ♂	T. claytoni ♀
T. claytoni ♀	T. claytoni ♀
T. claytoni ♂	T. claytoni ♂

All combinations of nematodes swarmed together, and when small groups of 10-15 nematodes of each species were mixed, there was mutual swarming of the 2 species. There was no indication that swarming was species specific between T. martini and T. claytoni, as had been found for mixtures of swarming T. martini and the Tylenchorhynchus sp. from Grand Isle, Louisiana. This test could not be repeated at a later date because the swarming condition did not persist in populations of T. claytoni in greenhouse cultures.

Duration of Swarming in T. Martini after Removal of Host Plants

Populations of T. martini apparently reverted to a nonswarming condition as a result of decline in numbers in the absence of host plants, but where populations were maintained, the swarming condition persisted. Swarming was found after 9 months in 2 replications each of moist and dry soil treatments. Nematodes in 1 replication of the moist treatment were found to form swarms after 12 months. In a few instances, adults and larvae were found swarming together. Movement of the nematodes at the beginning of the experiment was rapid and the sticking was strong. In contrast to this, nematodes found in small swarms at the end of the experiment were sluggish in their movements, and the stickiness between individuals was weak.

After 12 months, 10-inch pots were filled with soil from each replication of the 2 treatments and were planted with 1 rice seedling so that nematode numbers could be maintained in the swarming condition. However, this attempt was not successful and samplings of nematodes after 4 months indicated that populations had declined markedly. This was attributable to low temperatures in the greenhouse.

Induction of Swarming

Effects of Complete Nutrition of the Host

Five host-nutrition tests involving nutrient solutions and rice grown in sand were conducted to evaluate the influence of complete

host nutrition on the induction of swarming. Two problems that limited experiments of this type involved the survival and growth of rice in culture and the survival and reproduction of the nematodes. Rice is a difficult plant to work with in nutrient sand cultures, and growth must be maintained for at least 45 days to allow nematodes to swarm. In addition, nematode populations are difficult to build up under such conditions.

Test I consisted of 4 treatments and 4 replications set up in a randomized block design (Table 3).

Table 3. Populations per pint of sand of Tylenchorhynchus martini Fielding, 1956 cultured on rice for 60 days, with indications where swarming occurred.

Treatment	Rep A	Rep B	Rep C	Rep D
Low potassium	28	0	0	0
Low phosphorus	0	0	0	0
Low nitrogen	112	0	126	1442
Complete	3150[a]	84	2625[a]	1400

[a]Swarms present

Plant growth was poor in the phosphorus and potassium-deficient treatments, while growth in the nitrogen-deficient treatments was somewhat better. Treatments receiving complete nutrient applications exhibited vigorous growth in 3 of 4 replications. One replication of

nitrogen-deficient plants was growing as well as the poorest growth in the complete nutrient replications.

Populations of nematodes did not survive in phosphorus and potassium-deficient treatments. Nitrogen-deficient treatments supported increases in populations in 3 of 4 replications. Complete nutrient treatments yielded relatively high numbers in 3 replications and a moderate increase in the fourth replication. Large swarms were found in 1 replication and small swarms in another, while no evidence of swarming was observed in the 2 replications containing lower populations.

Tests II and III were discontinued after 45 days due to the lack of nematode survival and plant growth.

Test IV was conducted to study the effects of minor nutrient elements. This test consisted of 11 treatments with 3 replications in a randomized block design. Nematode samples were taken from 1 replication of each treatment 60 days after inoculation to determine whether the population was in a swarming condition. Before extraction of nematodes, each sample of sand was adjusted to 250 ml; however, nematode numbers were calculated in terms of a pint of sand (473 ml). Swarming was not found in this test after 60 days, although there were marked increases in T. martini populations. The experiment was sampled again after 75 days and swarming was observed in 2 replicates of the complete nutrient solution and 2 replicates of the

complete nutrient solution plus asparagine (Table 4). Nematode numbers and plant growth were good in each replication where swarming was found. Large swarms were formed in 1 replicate of the complete nutrient plus asparagine and small swarms were found in another replicate of this treatment.

Test V was conducted as a repetition of Test IV and consisted of 10 treatments in triplicate. Samples were taken from 1 replication in each treatment after 45 days, and although there was no swarming, populations increased markedly. At the termination of the test after 60 days, all replications were sampled. Swarming was detected in 1 replicate each of the complete nutrient treatment plus casein hydrolysate and the sulfate-deficient treatment (Table 5). Nematode numbers were high in the complete nutrient plus casein hydrolysate and the sulfate-deficient treatment (Table 5). Nematode numbers were high in the complete nutrient plus casein hydrolysate treatment, but were much lower in the sulfate-deficient treatments, and these numbers were correlated with plant growth.

Effect of Nitrogen Level

Rapid growth and development of the host plant has been associated with induction of swarming in T. martini (14). Increases in growth of rice plants resulted when nitrogen rates were shifted from 10 to 320 ppm (15). Since these results indicate a nitrogen influence on plant growth, tests for induction of swarming were

Table 4. Populations of Tylenchorhynchus martini Fielding, 1956 per pint of sand from rice nutrition Test IV sampled at 75 days, with indications where swarms occurred.

Treatment	Replication 1	2	3
Complete -Fe	2758	3535	756
Complete	2380[a]	3864	4091[a]
Complete + Casein hydrolysate	532	2625	1743
Complete + Asparagine	2485[a]	3409[a]	2751
Complete + Glutamine	2632	1512	1967
-Nitrogen	1176	427	728
-Phosphorous	686	525	413
-Potassium	2947	2408	1694
-Sulfate	2282	840	1148
-Calcium	2632	1078	812
-Magnesium	609	245	77

[a]Swarms found

Table 5. Populations of Tylenchorhynchus martini Fielding, 1956 per pint of sand in each replicate and treatment of nutrition Test V, 60 days after addition of nematodes, with indications where swarms occurred.

Treatment	Replication 1	2	3
Complete -Iron	56	406	1267
Complete	1708	2156	1148
Complete + Asparagine	861	1463	994
Complete + Casein hydrolysate	588	3430[a]	2457
-Nitrogen	2527	357	763
-Phosphorous	609	1281	343
-Potassium	84	105	294
-Sulfate	490[a]	133	896
-Magnesium	2261	350	280
-Calcium	1498	728	378

[a]Swarms found

conducted on rice and sugarcane in a complete nutrient medium supplied with different levels of nitrogen. These tests were set up on greenhouse benches with 5 treatments and 4 replicates (Table 6).

Table 6. Design of tests for effects of nitrogen levels supplied to rice and sugarcane on populations and swarming of Tylenchorhynchus martini Fielding, 1956.

Treatment number	Reps	Lbs. fertilizer per acre			Lbs. fertilizer per acre		
		N	P	K	N	P	K
1	4	180	40	40	270	60	100
2	4	60	40	40	90	60	100
3	4	20	40	40	30	60	100
4	4	0	0	0	0	0	0
5[a]	4	60	0	40	90	60	0

[a]The fertilizer mixtures in treatment 5 were without phosphorus for rice and potassium for sugarcane.

Six weeks after the start of this test, 1 replicate of each treatment with the most vigorously-growing rice plants was selected for sampling. Pots selected were sampled at 6, 10, 12, and 16 weeks. Five additional pots were sampled at the end of 17 and 19 weeks, at which time swarming was detected in 1 replicate.

Strong swarms were noted at 19 weeks in the rice test in only 1 replicate of treatment 4. Small swarms had been noted at 16 and 17 weeks in this replicate when 2 to 3 nematodes showed some indication of swarming by slight stickiness and jerky movements.

There was no evidence of swarming in samples taken after 20 weeks from the sugarcane experiment. Nematode numbers were considered high enough for swarming to occur, but slowness of the population buildup during the first 8 weeks may have been the limiting factor. Numerous species of free-living nematodes were found in all samples.

DISCUSSION

Swarming of nematodes suspended in water and water solutions of chemicals results from mechanical contact between individuals possessing a sticky condition of the cuticle. Three-dimensional masses are formed by the activity of the nematodes possessing the cuticular stickiness. The nematode activity is best described as continual and rapid, jerky movements which result apparently from attempts of individuals to break free from the swarms. Nonswarming nematodes as well as mineral debris present in the suspension are expelled from the aggregates of swarming nematodes.

Anabiosis or dormancy is a phenomenon of nematode aggregation which has been the object of several investigations since Needham's (19) report in 1744 on revival of dormant Tylenchus tritici (Anguina tritici) (Steinbeck, 1799) Filipjev, 1936. Nematodes in the anabiotic or dormant state are able to endure more extreme temperatures than active specimens (2, 4, 21). Induction of dormancy under natural conditions has been associated with unfavorable effects, including low and high temperatures, desiccation, and low food supply (6, 26). Revival of dormant nematodes to the active state has been found dependent upon oxygen tensions in water suspensions (8). Natural environmental conditions favoring nematode growth and reproduction are effective in reviving dormant nematodes.

Anabiosis provides a means whereby nematodes may survive unfavorable conditions through extended periods without appreciable harm. Fielding (5) revived dormant A. tritici after a period of 28 years. In contrast to this, Birchfield (1) found the longevity of non-dormant Radopholus similis (Cobb, 1893) Thorne, 1949 in the absence of host plants to be less than 4 months.

There are several important points that distinguish anabiosis and swarming. The two phenomena represent extremes in nematode activity. Anabiosis is a dormant or inactive state, and swarming is a state of exaggerated mobility. Anabiosis occurs predominantly in preadult nematode stages, whereas swarming involves all nematode stages. The ability to enter an anabiotic state of more or less extended duration is known in members of 10 to 12 of about 80 established families of nematodes. The condition prevails in certain soil-inhabiting and in moss-inhabiting nematodes, and is unknown in marine species and in the Dorylaimoidea (25).

Swarming embraces a wider range of nematode forms including some in the Dorylaimoidea. Swarming is characterized by innate cuticular stickiness, which is due apparently to a morphological modification of the exocuticle, or cuticle surface layer in non-swarmers. By contrast, aggregations of dormant nematodes exhibit no stickiness. According to Steiner (26), masses of dormant nematodes occur because the individuals are held together by viscous

secretions of their esophageal glands, after these substances are expelled through the buccal apparatus and cover the external surface of the nematodes.

Evidence obtained in previous work indicates that the capacity to enter a physiological state of swarming is under genic control (11). Swarming was species specific between T. martini and an undescribed species of Tylenchorhynchus from Grand Isle, Louisiana. Participation of both sexes of the undescribed species in swarms indicated the reaction was nonsexual. Tests during the present work involving swarming in T. martini and T. claytoni resulted in a reaction between these two species. Swarming in T. claytoni was also nonsexual. A possible explanation for a reaction involving T. martini and T. claytoni is that group specificity exists within the genus Tylenchorhynchus.

An interesting observation made during the course of the longevity test was that all stages of T. martini, including juveniles, swarmed with adult nematodes. The fact that this occurred several months after the removal of host plants indicated that the capacity to swarm is transmitted to the offspring.

The idea of a genic mechanism for control of the ability of nematodes to undergo induction of swarming arises from facts which suggest that the nature of cuticular stickiness is innate--meaning that it arises from structural modifications of the cuticle surface layer, brought about by secretions from the underlying hypodermis.

These facts include induction and duration of swarming; its independence of environmental factors in vitro such as pH and drastic chemical treatments, and its inhibition by crystalline trypsin.

Investigation of host-plant mineral nutrition and its relation to the induction of swarming in T. martini indicates that complete nutrition of the host is necessary. Nutrient supplements such as casein hydrolysate, asparagine, and glutamine do not appear to hinder or expedite induction of swarming.

Results of the swarming longevity tests indicate that swarming can be maintained through adverse conditions for long periods of time if a sufficient portion of the ancestral population survives.

Only one hypothesis on the significance of swarming in the life of nematodes has been advanced. Meyl (18) observed swarming in H. typica, called it "Nesterbildung," and reported that it played a sexual function in the life of this nematode species. The fact that swarming occurs also in nematode species without males and is independent of sex in swarming reactions where both sexes are present, indicates however, that a more fundamental need or advantage in the life of nematodes must be involved.

The induction of swarming by rapidly growing host plants and by complete nutrition of the host in greenhouse tests suggests that the phenomenon is the result of nematode population response to superabundant food levels. Once swarming occurs, or has been

induced in a population, the condition persists for an indefinite period in the absence of food. The phenomenon apparently reflects the utilization of excess food and results from a physiological transformation involving associated changes in the nematode body. Aggregation in masses serves to keep individuals of a species together during a period of food shortage. Exposure of swarms to lower food levels brought about by a seasonal resumption of host-plant root growth, following either a period of abundant food or a shortage of food, results in a reversion to a nonswarming condition of the progeny feeding on these lower food levels, and to their dispersion in the soil.

SUMMARY

Swarming, as it occurs in aqueous solutions, is characterized by the exaggerated activity of masses of nematodes possessing a stickiness of the cuticle. The weight of evidence suggests that this stickiness results from an innate modification of the structure of the exocuticle. Swarming has been observed in a wide spectrum of nematode types, embracing 13 species from a variety of hosts, environments and geographical locations.

The phenomenon of swarming in water suspensions is dependent upon time, density, and activity of the nematodes and is independent of light, sex, stage of nematode development, pH, and chemical treatments in water suspensions, except where nematode viability and mobility are affected. The phenomenon is also independent of common soil environmental factors, such as soil type, fertility, and moisture level.

Mixed populations of Tylenchorhynchus martini Fielding, 1956 and T. claytoni Steiner, 1937 swarmed, indicating a nonspecific or group reaction, in contrast to separate swarming (species specific reaction) for T. martini and an undescribed species of Tylenchorhynchus from Grand Isle, Louisiana.

Greenhouse-bred populations of T. martini swarmed after their culturing for 60-75 days on rice fertilized with complete mineral

nutrient solutions. Organic supplements to nutrient solutions had no effect on the induction of swarming. Swarming of T. martini did not occur under conditions of incomplete nutrition of rice or sugarcane.

LITERATURE CITED

1. Birchfield, W. 1957. Observations on the longevity without food of the burrowing nematode. Phytopathology 47:161-162.

2. Bosher, J. E. and W. E. McKeen. 1954. Lyophilization and low temperature studies with the bulb and stem nematode Ditylenchus dipsaci (Kuhn 1858) Filipjev. Proc. Helm. Soc. Washington, D. C. 21:113-117.

3. Chapman, R. A. 1958. Personal communication.

4. Courtney, W. D. and R. Latta. 1934. Some experiments concerning the revival of quiescent Anguillulina dipsaci. Proc. Helm. Soc. Washington, D. C. 1(1):20-21.

5. Fielding, M. J. 1951. Observations on the length of dormancy in certain plant infecting nematodes. Proc. Helm. Soc. Washington, D. C. 18:110-112.

6. Filipjev, I. N. and J. H. S. Stekhoven, Jr. 1941. A manual of agricultural helminthology. E. J. Brill. Leiden, Holland. 878 pp.

7. Gallegly, Jr., M. E. and J. C. Walker. 1949. Plant nutrition in relation to disease development. V. Bacterial wilt of tomato. Am. Jour. Bot. 36:613-623.

8. Hastings, R. J. and W. Newton. 1934. The influence of a number of factors upon the activation of dormant or quiescent bulb nematodes, Anguillulina dipsaci (Kuhn, 1858) Gerv. and v. Ben., 1859 (Anguillulinidae). Proc. Helm. Soc. Washington, D. C. 1(2): 52-54.

9. Hollis, J. P. 1958. Induced swarming of a nematode as a means of isolation. Nature 182:956-957.

10. _____. 1960. Mechanisms of swarming in Tylenchorhynchus species (Nematoda, Tylenchida). Abst. Phytopathology 50: 639-640.

11. _____. 1962. Nature of swarming in nematodes. Nature 193:798-799.

12. Hollis, J. P. 1962. A survey of plant parasitic nematodes and their control in Kenya. FAO Plant Protection Bulletin 10(5): 97-106.

13. _____. 1963. Personal communication.

14. _____ and J. M. McBride. 1962. Induction of swarming in Tylenchorhynchus martini (Nematoda, Tylenchida). Abst. Phytopathology 52:14.

15. Hoshino, T., S. Samoto, and K. Outi. 1958. Influence of nitrogen upon the various characters of two types of rice varieties cultured in nutrient solution. Jap. Jour. of Breeding. 8:155-164.

16. Johnson, C. M., P. R. Stout, T. C. Broyer, and A. B. Carlton. 1957. Comparative chlorine requirements of different plant species. Plant and Soil 8:337-353.

17. Lees, E. 1958. Personal communication.

18. Meyl, A. H. 1955. Ueber ein seltenes massenauftreten der pflanzenparasitischen Hemicycliophora typica deMan, 1921 (Nematoda, Criconematidae) sowie erganzungen zu ihrer beschreibung. Nachr. Bl. Dtsch. Pfl. Schutz Dienstes. 7:1-3.

19. Needham, T. 1744. A letter concerning certain chalky tubulous concretions, called malm: with some microscopical observations on the farina of the red lily and of worms discovered in smutty corn. Philos. Trans. Roy. Soc. 42:634-641.

20. Seinhorst, J. W. 1957. Personal communication.

21. Sherman, Grace W. 1934. Survival and revival of Anguillulina dipsaci from narcissus material. Proc. Helm. Soc. Washington, D. C. 1(1):19-20.

22. Staniland, L. N. 1957. The swarming of Rhabditid eelworms in mushroom houses. Plant Pathology 6:61-62.

23. Steinberg, R. A. 1935. Nutrient solution purification for removal of heavy metals in deficiency investigations with Aspergillus niger. Jour. Agri. Res. 51:413-424.

24. Steiner, G. 1940. Anabiosis in nematodes, its distribution, mechanism and significance. Proc. Third Inter. Cong. Microbiol. New York. 334-335.

25. Steiner, G. 1953. The zoological and agricultural status of plant nematodes. Proc. Fourteenth Intern. Cong. Zool. Copenhagen. 368-371.

26. _____. 1960. Personal communication.

27. Stout, P. R. and D. E. Arnon. 1939. Experimental methods for the study of the role of copper, manganese, and zinc in nutrition of higher plants. Am. Jour. Bot. 26:144-149.

28. Wallace, H. R. 1961. The bionomics of the free living stages of zoo-parasitic and phyto-parasitic nematodes-a critical survey. Helm. Abst. 30(1):1-22.

29. Whitehead, A. G. 1960. Proceedings of the First Inter-African Plant Nematology Conference. East African Agri. and For. Res. Org. Kikuyu, Kenya. 32 pp. (Mimeographed).

VITA

Jere M. McBride was born September 5, 1937 at Weston, a community 6 miles east of Jonesboro, Louisiana. He was graduated from high school in May, 1955 and entered Louisiana Polytechnic Institute in June, 1955. He completed the requirements for the Bachelor of Science degree in Wildlife Conservation and Game Management in June, 1959. After receiving his B. S. degree he entered Louisiana State University in June, 1959. He completed the requirements for the degree of Master of Science in the Department of Botany, Bacteriology and Plant Pathology in January, 1961. Upon receiving his M. S. degree he continued his graduate program at Louisiana State University. On September 15, 1962 he was married to the former Brenda Williams of New Orleans, Louisiana. He is a candidate for the degree of Doctor of Philosophy in the Department of Botany and Plant Pathology in January, 1964.

EXAMINATION AND THESIS REPORT

Candidate: Jere M. McBride

Major Field: Plant Pathology

Title of Thesis: Studies on the Induction of Swarming in Tylenchorhynchus martini Fielding, 1956 (Nematoda, Tylenchida)

Approved:

John P. Hollis Jr.
Major Professor and Chairman

Max Goodrich
Dean of the Graduate School

EXAMINING COMMITTEE:

John P. Hollis Jr.

M. T. Henderson

Wm Burchfield

Louis Anastasiow, Jr.

J. L. Forbes

Date of Examination:

January 10, 1964